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Dr. Faith N. Brenneman
Department of Biochemistry
Yale University
333 Cedar Street
New Haven, Connecticut

Dear Faith,

Sounds as though you will be busy filling out forms for awhile. I'll try to help out by suggesting several projects. You are free to use any of the ones mentioned and I would certainly urge you to read up some and expand the brief outline I will offer when you finally do write up the applications. Of course, it is entirely possible that the outcome of our current work, or your own interests, may change the final decision when you actually get to work but we needn't worry about that. Now for some suggestions.

1. To study the biosynthesis of 5-ribosyl uracil 5'-phosphate (also known as pseudouridylic acid). We have done an in vivo experiment, with growing yeast, showing that the pseudouridylic in RNA comes directly from orotic. An almost identical experiment was published by Hall and Allen, *Biochim. et Biophys. Acta*, 39, 557 (1960). Subsequently, we spent a fair amount of time trying to get an in vitro system going, using, primarily, yeast extracts and going on the hunch that the reaction might be, overall, $\text{orotic} + \text{PRPP} \longrightarrow \text{5-ribosylorotic-5'-monophosphate} + \text{PP}$ $\text{5-ribosylorotic-5'-monophosphate} \longrightarrow \text{5-ribosyluracil-5'-monophosphate}$. We were completely unsuccessful. One of the problems is to work out a decent assay for 5-ribosyluracil in the presence of uridylic, since under our conditions our extracts, of course, made a lot of uridylic. The spectrum of 5-ribosyluracil was not sensitive enough, nor the odd orcinol reaction (for both, see W. Cohn, *J. Biol. Chem.*, this summer sometime). We finally resorted to paper chromatography and labeled orotic. It is entirely possible that the reaction is not as written above. How about $\text{UMP} + \text{PRPP} \longrightarrow \text{5-ribosyl-5'-monophosphate-5'-uridylic acid} + \text{PP}$ $\text{5-ribosyl-5'-monophosphate-5'-uridylic acid} \longrightarrow \text{5'-pseudo-uridylic acid}$. This should be looked into, perhaps by starting with reaction 2. Perhaps we could try our hand at making that diribose-uracil derivative chemically, starting with pseudouridine, which is readily isolable from S-RNA.

2. To study kinetics and mechanism of action of polynucleotide phosphorylase. Have a look at enclosed reprints and see how much really needs to be done--Kms, the relation between the three reactions, exchange, polymerization, phosphorolysis. The kinetics of any polymerization reaction is fascinating. Before I start to write a book on what's to be done, I'll stop. One of the chief obstacles to doing this has been the lack of a good preparation, but I hope this will be solved before you get here. We have started afresh with M. lysodeikticus and now have a rather crude preparation which has, however, the lovely attribute of being almost free of nucleic acid and has an absolute primer requirement. It should be ready for detailed mechanism experiments when you get here. By the way, look at Olmsted's papers on this enzyme, J.B.C., 234, 2965, 2971 (1959). Our data, so far, does not support his idea of different enzymes for different diphosphates. Our activities go along with one another and we have not been able to repeat his specific inhibitions. This problem would probably be more sure-fire than #1, but the other might be more interesting to you. This would offer you the opportunity to learn to make polymers, oligonucleotides, etc.

3. Another problem already begun is the study of an interesting nuclease in Ehrlich Ascites Tumor. Malcolm Martin, a third-year Yale medical student has worked on this summers, but this coming summer will be his last and you could take it up. It will need more purification as a start. Also, specificity studies on specific polymers as well as on RNA. It seems to be fairly similar to the leukemia enzyme described by Anderson and Heppel (see enclosed reprint). We have some preliminary experiments indicating it gives interesting oligonucleotides from S-RNA and this would be a particular area to push. It would no doubt get you into sequence studies with S-RNA in an investigation of the products and would be very instructive as well as a lot of fun. We could probably use some new column techniques for separating oligonucleotides (DEAE, etc.) as well as paper chromatography and electrophoresis. Also, various specific chemical and enzymic procedures for studying the structures. (See Heppel's review, enclosed).

When you make a decision let me know and I'll come up with more details. Best of luck on the applications. I certainly hope that this all works out as we would enjoy having you here.

Yours,

Enclosures

Maxine Singer

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